Data Evaluation Report on the acute toxicity of ETU to Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 45910401

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D289573
OECD Data Point	
EPA MRID	45910401
EPA Guideline	72-1(c)

Test material: Ethylenethiourea
Common name: ETU (Reg. No. 146099)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 96-45-7
Synonyms: Not reported

Purity: 99.9%

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 6/30/03

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Secondary Reviewer(s):
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Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 600016

Date Evaluation Completed:

CITATION: Zok, S. 2001. Reg. No. 146099- Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours). Unpublished study performed by BASF Akiengesellschaft, Experimental Toxicology and Ecology, Ludwigshafen/Rhein, Germany. Laboratory Project Identification No. 12F0533/005042 (BASF Reg. Doc. No. 2001/1001877). Study sponsored and submitted by EBDC/ETU Task Force (BASF Corporation, Research Triangle Park, NC; Cerexagri, Inc., King of Prussia, PA; Dow AgroSciences LLC, Indianapolis, IN; and Griffin LLC, Valdosta, GA). Experimental start date November 6, 2000 and experimental termination date November 10, 2000. The final report issued February 15, 2001.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to ETU at mean measured concentrations of 21.6, 49.9, 100.7, 218.7, and 502.0 mg/L under static conditions. The nominal concentrations were 0 (negative control), 22, 50, 100, 220, and 500 mg/L. After 96 hours of exposure, 10% mortality was observed in the 502 mg/L treatment group. No other mortalities were observed. At 48 hours, one fish was observed tumbling in the 502 mg/L treatment group. No other sublethal effects were observed. The LC_{50} was >502 mg/L, which categorizes ETU as practically non-toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC was 219 mg/L.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (72-1c). This study is classified as CORE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 1.0 g, 50 mm
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: >502 mg/L 95% C.I.: N/A
NOEC (mortality and sublethal effects): 219 mg/L

Endpoints affected: mortality and sublethal effects

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the EPA guideline, Pesticide Assessment Guidelines, subdivision E, Hazard Evaluation Wildlife and Aquatic Organisms, U.S. Environmental Protection Agency, Washington DC, para. 72-1 (1982); EPA-SEP (Standard Evaluation Procedure) No. 540/9-85-006 (1985); EEC directive 92/69, Annex V, C1; and OECD Guideline No. 203("Fish, Acute Toxicity Test" (1992). Deviations from §72-1c included:

1) The hardness (250 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH range (8.2-8.6) was greater than recommended (7.2-7.6). The oxygen content in terms of percent saturation was not reported.

2) The dilution water was non-chlorinated, charcoal-filtered and aerated, tap water. The US EPA recommends against using dechlorinated tap water.

These deviations do not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. The test was conducted in accordance with the GLP provisions of the "Chemicals Act" (Chemikaliengesetz, Germany) and the OECD Principles of Good Laboratory Practice (Paris, 1981).

A. MATERIALS:

1. Test Material ETU (Reg. No. 146099)

Description: White solid (crystalline)

Lot No./Batch No. : 01743-136

Purity: 99.9%

Stability of Compound Under Test Conditions: The 0 hour test solutions were 97.7-102.3% of nominal and the 96 hour test solutions were 99.0-103.1% of nominal. Results are presented in Tables 1 and 3, pp. 10-11 of the Analytical Report.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. All OECD requirements were not reported.

Water solubility: 19.3 g/L

Storage conditions of test chemical: Stored at room temperature.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792)

Age at test initiation: Approximately 4 months

Weight at test initiation: 1.0 g (0.8-1.4 g)

Length at test initiation: 50 mm (47-55 mm)

Source: Forellenhof Fredelsloh, Moringen, Germany

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A non-GLP range-finding study was conducted. The LC_{50} after 96 hours was >500 mg/L. The definitive nominal test concentrations were determined based on the range-finder results.

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	14 days prior to testing.	
Conditions: (same as test or not)	Same as test	
Feeding:	Growing feed (Forellenfutter, Zeigler), *ad libitum*, with live and frozen brine shrimp (artemia) on workdays, except during the last day prior to testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	0% mortality during the last 7 days of acclimation.	
Duration of the test	96 hours	*EPA/OECD requires: 96 hours*

Parameter	Details	Remarks Criteria
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	The dilution water was aerated prior to testing. No aeration during testing.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass with stainless steel frame 60 x 35 x 40 cm 25 L (depth of ~12 cm)	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*

Parameter	Details	Remarks Criteria
Source of dilution water	The dilution water was non-chlorinated, charcoal-filtered and aerated, tap water.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water; OECD permits dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 250 mg $CaCO_3$ /L 8.2-8.6 7.3-10.3 mg/L Not reported Not reported Not reported Not reported Not reported 11-12°C N/A DO, pH, and temperature were determined daily. Additionally the temperature was measured hourly in one test aquaria.	The hardness (250 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH range (8.2-8.6) was greater than recommended (7.2-7.6). The oxygen content in terms of percent saturation was not reported. The test water was regularly assayed for chemical contaminants and microbes.

Parameter	Details	Remarks Criteria
		Hardness and pH *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative control), 22, 50, 100, 220, and 500 mg/L . ND (not detected, negative control), 21.6, 49.9, 100.7, 218.7, and 502.0 mg/L.	The mean measured concentrations were reviewer-calculated from the 0, 48, and 96 hour measured concentrations. *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	N/A	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent , exceed 100 mg/L.*
Number of fish/replicates: negative control: solvent control: treated:	20 fish, 10 fish in two test vessels N/A 20 fish, 10 fish in two test vessels	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.4 g fish/L	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark.	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*

Parameter	Details	Remarks/Criteria
		Criteria
Recovery of chemical Level of Quantitation Level of Detection	96.9-103.1% 0.2 mg/L Not reported	Based on analytical recoveries from the 0, 48, and 96 hour samples.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	Every 24 hours.	*EPA/OECD requires: minimally every 24 hours*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, 10% mortality was observed in the 502 mg/L treatment group. No other mortalities were observed.

Table 3: Effect of ETU on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, mg/L, measured and (nominal conc.)[a]	No. of fish at start of study	0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	0	0	0	0	0	0
22 (22)	20	0	0	0	0	0	0

50 (50)	20	0	0	0	0	0	0
101 (100)	20	0	0	0	0	0	0
219 (220)	20	0	0	0	0	0	0
502 (500)	20	0	0	2	10	2	10
NOEC (mortality)	219 mg/L						
LC_{50} (95% C.I.)	>502 mg/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are in parentheses.

B. NON-LETHAL TOXICITY ENDPOINTS:

At 48 hours, one fish was observed tumbling in the 502 mg/L treatment group. No other sublethal effects were observed.

Table 4. Sublethal effects of ETU on Rainbow trout (*Oncorhynchus mykiss*).

Treatment, mg/L, measured and (nominal conc.)[a]	**endpoint at 24 Hours**	**endpoint at 48 Hours**	**endpoint at 72 Hours**	**endpoint at 96 Hours**
	% affected	**% affected**	**% affected**	**% affected**
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
22 (22)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
50 (50)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
101 (100)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
219 (220)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
502 (500)	No abnormalities detected	Tumbling (5%)	No abnormalities detected	No abnormalities detected
NOEC (sublethal)	219 mg/L			
LOEC (sublethal)	502 mg/L			

Treatment, mg/L, measured and (nominal conc.)[a]	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
EC_{50}	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are in parentheses.

C. REPORTED STATISTICS:

Statistical Method: The 96-hour LC_{50} value was estimated (value of interpolation). The NOEC was determined based on mortality and sublethal effects data. The results were based on mean measured concentrations.

LC_{50}: >502 mg/L 95% C.I.: N/A
NOEC (mortality and sublethal effects): 219 mg/L

Endpoints affected: mortality and sublethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} and NOEC could be visually determined.

96-Hour
LC_{50}: >502 mg/L 95% C.I.: N/A
NOEC (mortality and sublethal effects): 219 mg/L

Endpoints affected: mortality and sublethal effects

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-1c that affected the acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. The LC_{50} was >502 mg/L, which categorizes ETU as practically non-toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

The test material was visually present as dispersion in the nominal 500 mg/L treatment group. The other treatment solutions (≤220 mg/l) were clear. All treatment group solutions were prepared one day before test initiation.

G. CONCLUSIONS:

This study is scientifically sound and fulfills U.S. EPA guideline §72-1, and is classified as CORE. The NOEC was 219 mg/L. The LC_{50} was >502 mg/L, which categorizes ETU as practically non-toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

96-Hour
LC_{50}: >502 mg/L 95% C.I.: N/A
NOEC (mortality and sublethal effects): 219 mg/L

Endpoints affected: mortality and sublethal effects

III. REFERENCES:
No references were cited.